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Contribution of nitrogen fixation to the external nitrogen load of a water quality control reservoir

(Kis-Balaton Water Protection System, Hungary)

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## Abstract

To reduce external nutrient loading by the greatest nutrient transporter to Lake Balaton, the Kis-Balaton Water Protection System commenced operation in 1985. Cyanobacterial blooms (*Cylindrospermopsis raciborskii*, *Aphanizomenon sp.* and *Anabaena sp.*) cause nitrogen loading via nitrogen fixation, which can exceed the total external N-load to the reservoir during the summer. Nitrogen fixation of phytoplankton in the system in 2009 was measured using the  $^{15}\text{N}$ -isotope technique. The light dependence of fixation was described by an exponential saturated curve which was then used to calculate daily nitrogen fixation. The total amount of nitrogen fixed during summer months (July-August) exceeded 170 tons for the 21 km<sup>2</sup> of open water area, which was more than three times higher than the external load by inflows. Total nitrogen: total phosphorus mass ratios (2.5–11.5) favoured the development of a N<sub>2</sub>-fixing cyanobacterial bloom, the contribution of which to the total algae biomass varied between 50 and 90%. The estimated primary production of phytoplankton was used to calculate the contribution of nitrogen fixation (0.01–28%) to algae nitrogen demand. Nitrogen fixing cyanobacteria were found to play a key role in addressing the nitrogen demand of phytoplankton.

Keywords: cyanobacteria, N<sub>2</sub>-fixation,  $^{15}\text{N}$ -methodology, nutrient ratio, shallow water, water quality control reservoir.

## Introduction

Lake Balaton is of international significance as an environmental resource, and is one of the most important touristic and recreational regions of Hungary. Of even greater importance is its utilization for drinking water, particularly during summer months. The Kis-Balaton Water Protection System (KBWPS) was established to improve and protect the water quality of Lake Balaton, primarily through phosphorus retention (Pomogyi, 1991). The KBWPS also serves as a nature protection area, and the water is not intended as a drinking water supply. Nutrient retention by the KBWPS was especially crucial prior to the implementation of other measures, which include the construction of a regional sewage treatment system designed to export P from the watershed (Szilágyi et al., 1990). The greatest inorganic nutrient transporter of the KBWPS is the Zala River, which flows through the reservoir and enters the westernmost basin of Lake Balaton (the Keszthely basin). The nutrient load of the Zala River is approximately one third to one half of the total external nutrient load to the lake (Szilágyi et al., 1990). At the end of the 1960s, water quality had severely deteriorated and eutrophication had accelerated in Lake Balaton (Pomogyi, 1993a). Joó & Lotz (1980) suggested that the nutrient load from the Zala River watershed may be retained by a wetland area prior to entering the lake. Approximately half of the inorganic

nutrients are diffusely sourced, and it would thus be impossible to remove them at their origin using traditional methods. Construction of the KBWPS began in 1981. Operation of the first part of the system, the Upper reservoir, began in 1985, and the second part, the Lower reservoir, was partially flooded in 1992. After many studies of nutrient retention and removal mechanisms, it was found that the efficiency of the system is generally good (Pomogyi, 1993b). During the first years, the total nitrogen load decreased by half and the total phosphorus load declined by 10–15%. This temporary period of nitrogen depletion favoured the development of a N<sub>2</sub>-fixing cyanobacterial bloom, which caused internal N-loading (, Szilágyi et al., 1990). Over the last 15 years, the total nitrogen load has fluctuated greatly (<http://www.kisbalaton.hu>, 2012), and despite the intensive nitrogen fixation by cyanobacteria, it appears to have decreased from 900 to 200 tons N. The total phosphorus load of the Zala River at the end of the twentieth century had decreased to one-tenth the load of the mid-1980s, mainly due to chemical P precipitation at the sewage treatment facility of Zalaegerszeg (Tátrai et al., 2000).

In aquatic ecosystems, N and P most frequently limit primary production. The availability of these macronutrients and their low supply ratios favour the development of cyanobacterial blooms (Hall et al., 2005). Many cyanobacteria of the order Nostocales are able to fix nitrogen, and thus outcompete other species in nitrogen-poor environments (Huisman & Hulot, 2005). Cyanobacteria species usually dominate in lakes characterized by low N:P ratios. In 1986 cyanobacteria proliferated in the KBWPS and the resulting amount of nitrogen fixed exceeded the total external N load during the summer (Gorzó, 1990). Kovacs (2002) found nitrogen fixation to be as high as 10% of the total annual N-load.

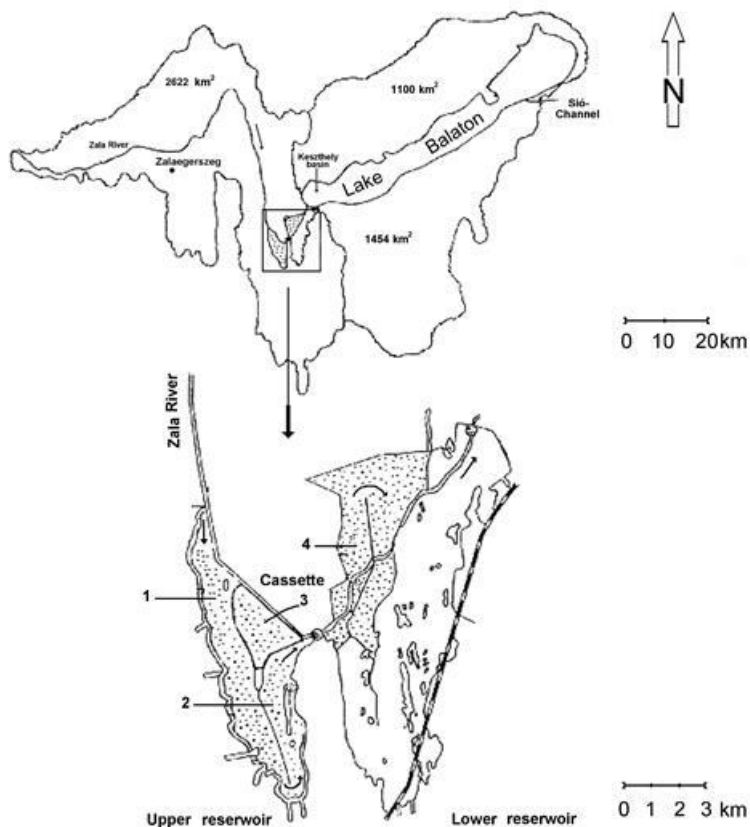
The aims of this study were (1) to calculate the contribution of nitrogen fixation to the external nitrogen load to the reservoir, (2) to estimate the contribution of nitrogen fixation by cyanobacteria to algae nitrogen demand in well-defined conditions (shallow, easily warmed, turbid water with high nutrient supply), and (3) to examine the effect of different environmental factors (primarily nutrient-supply) on nitrogen fixation. Several nitrogen fixation experiments were conducted using the acetylene reduction method at different locations in the reservoir (Kovács, 2002). However, the elapsed time since the last measurements (10 years), the disputable nutrient retention of the reservoir, and the possible problems with the acetylene reduction method were grounds for the current determination of the proportion of external N-load by cyanobacterial nitrogen fixation using the stable isotope (<sup>15</sup>N) technique.

## Materials and methods

### Study site

The Kis-Balaton Water Protection System is located in the valley of the lower Zala River, close to the western part of Lake Balaton (Fig. 1). The KBWPS has a 2,622 km<sup>2</sup> watershed, with one main (Zala River) and 16 small inflows. The outflow carries the water to the westernmost (Keszthely) basin of the Lake Balaton. The KBWPS consists of two main parts; the Upper and the Lower reservoirs. Due to their hydro-morphology and function, several water bodies of varying quality make up the system. The Upper reservoir has two sections; one of them is a large open water area (Hídvégi-Pond) with 18 km<sup>2</sup> of water surface, and the second is a smaller (3.5 km<sup>2</sup>) closed section without an outlet, called “Cassette”. The purpose of this section is to retain smaller floods or unexpected pollution from the Zala River (a detailed description can be found in Pomogyi, 1993b). The surface area of the Lower reservoir is 54 km<sup>2</sup> which is partially flooded and covered by marshlands and aquatic macrophytes over ~50% of its area. The main flow direction in the reservoir is regulated by dikes and sluices, which also determine the water retention time of the system (30 and 90 days in the Upper and the Lower reservoirs respectively). The mean depth is only 1.1 m, and the Secchi depth varies from 0.20 to 0.45 m.

The four selected sampling sites do not encompass the range of conditions across the whole reservoir, but were carefully chosen to characterise the four most typical and relatively large open water areas. These sampling sites correspond to the West-Transdanubian Environmental and Water Directorate (NyuDu-VizIg) regular sampling sites number 4, 7, 9, and 202. The first site is close to the mouth of the Zala River in the western part of the system (Korponai et al. 1997), the second is the largest open water area in the eastern part of the Upper reservoir, the third is in the Cassette, and the fourth is in the open water area of the Lower reservoir (Fig. 1). These sampling sites represent a total area of about 21 km<sup>2</sup> (4, 10.5, 3.5, and 3 km<sup>2</sup>, respectively).



**Fig.1** The four sampling sites of the Kis-Balaton Water Protection System (Hídvégi-Pond: 1) Pogányvári-víz, 2) Kányavári-víz; 3) Cassette; 4) Fenéki-Pond: Ingói-berek).

#### Analytical experiments and nitrogen fixation method

Water samples were taken with a 1.5 m sampling tube biweekly from July to October, 2009, and more frequently during the algae bloom. The nutrient concentrations of water samples were measured on the day of sampling. The determination of the dissolved N-forms – ammonium, nitrate and urea – were obtained following the methods of Mackereth et al. (1989), Elliott & Porter (1971) and Newell et al. (1967), respectively. The soluble reactive phosphorus (SRP) was determined as in Murphy & Riley (1962). The chlorophyll-*a* concentrations were measured according to Iwamura et al. (1970). The nutrient and chlorophyll-*a* concentrations were determined in three replicates, using a SHIMADZU UV-1601 spectrophotometer.

The determination of nitrogen fixation was followed after Présing et al. (2005). 100 ml water samples were measured into Duran Scott vessels (130 ml volume) and 1 ml 99.9 atom%  $^{15}\text{N}_2$  gas was injected. The water samples were incubated in the laboratory at lake temperature and illuminated at different irradiances (0, 35, 58, 108, 118, 207, 219, 343 and  $567 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), measured using a WALZ SQSA0156 LI-1400 data logger and stirred continuously. In preliminary experiments the optimal incubation time was determined to be 4 hours. After

incubation, 1 ml gas samples were taken from the gas phase of each incubation bottle, and the water samples were filtered through Whatman GF/C filters. The isotope enrichments and the nitrogen content of the gas and the solid samples were measured by an automated elemental analyser, interfaced with an Isotope Ratio Mass Spectrometer (ANCA-MS, Europa Scientific Ltd., U.K.). The  $^{15}\text{N}/^{14}\text{N}$  ratio of the gas samples was determined in triplicate and the N content and  $^{15}\text{N}/^{14}\text{N}$  ratios of the cells were determined in duplicate according to the methods of Pr  sing et al. (2001).

The light dependence of nitrogen fixation was described using the modified equation of Platt et al (1980). Daily nitrogen fixation was calculated from the global irradiation, the vertical light attenuation, and the light dependency parameters of fixation ( $V_{\text{max}}$ ;  $I_k$ ). The nitrogen fixation per unit surface area was calculated from the rates measured at optimal light intensity and the average depth of the water (Pr  sing et al., 1999).

Primary production of the algae was estimated using an empirical equation based on water temperature and chlorophyll-*a* concentration (V  r  s & V.-Balogh, 1997). The theoretical nitrogen demand of the phytoplankton was calculated from the primary production and the Redfield weight:weight ratio (C:N=5.68).

## Results

### Chlorophyll-*a* and nutrient concentrations

The chlorophyll-*a* concentrations of the four sites from July to October were between 85–370  $\mu\text{g L}^{-1}$  (Table 1). There were two chl-*a* maxima at each sampling site, one in July-August, and one in early autumn (August-September). The chl-*a* concentrations were much lower at the first and the third sampling sites (maxima of 221 and 245  $\mu\text{g L}^{-1}$ ) than at the second and fourth sites (maxima of 370 and 320  $\mu\text{g L}^{-1}$ ).

Ammonium concentrations were very low (around 15  $\mu\text{g L}^{-1}$ ) during the summer, except for at the fourth sampling site, where it exceeded several hundred  $\mu\text{g L}^{-1}$  in autumn, and at the first site at the end of August (120  $\mu\text{g L}^{-1}$ ). Ammonium concentrations in October significantly exceeded the summer concentrations at all sampling sites. The urea concentrations generally varied between 15–70  $\mu\text{g L}^{-1}$  at all sampling sites, with one exception in the middle of August. The nitrate concentrations exceeded 100  $\mu\text{g L}^{-1}$  in some cases (in September at the first and the third sampling sites), but otherwise fluctuated between 5–50  $\mu\text{g L}^{-1}$ . Generally, the urea concentrations were the predominant nitrogen forms in the summer.

The dynamics of the measured phosphorus compounds (total phosphorus (TP) and soluble reactive phosphorus (SRP)) were similar to those of the different N-forms (Table 1). Where algal biomass was highest (at the second and fourth sites), the available P concentrations remained low from July to October (< 10  $\mu\text{g L}^{-1}$ ). The Cassette,

the third site, is characterized by different water quality compared with the other parts of the reservoir, with the SRP sometimes reaching 300  $\mu\text{g L}^{-1}$  and the TP concentration exceeding 450  $\mu\text{g L}^{-1}$ .

The TN:TP mass ratios at all sampling sites ranged from 4.5 to 12, and rarely exceeded the Redfield mass ratio at the second and the fourth sampling sites (Table 1). The available N and P ratios were less than 7 at the first and third sampling sites, and fluctuated between 4 and 11 at the second one. The fourth sampling site was distinct; at the end of August, the ratio was greater than 30, and in October the ratio was greater than 90, owing to the high ammonium concentrations.

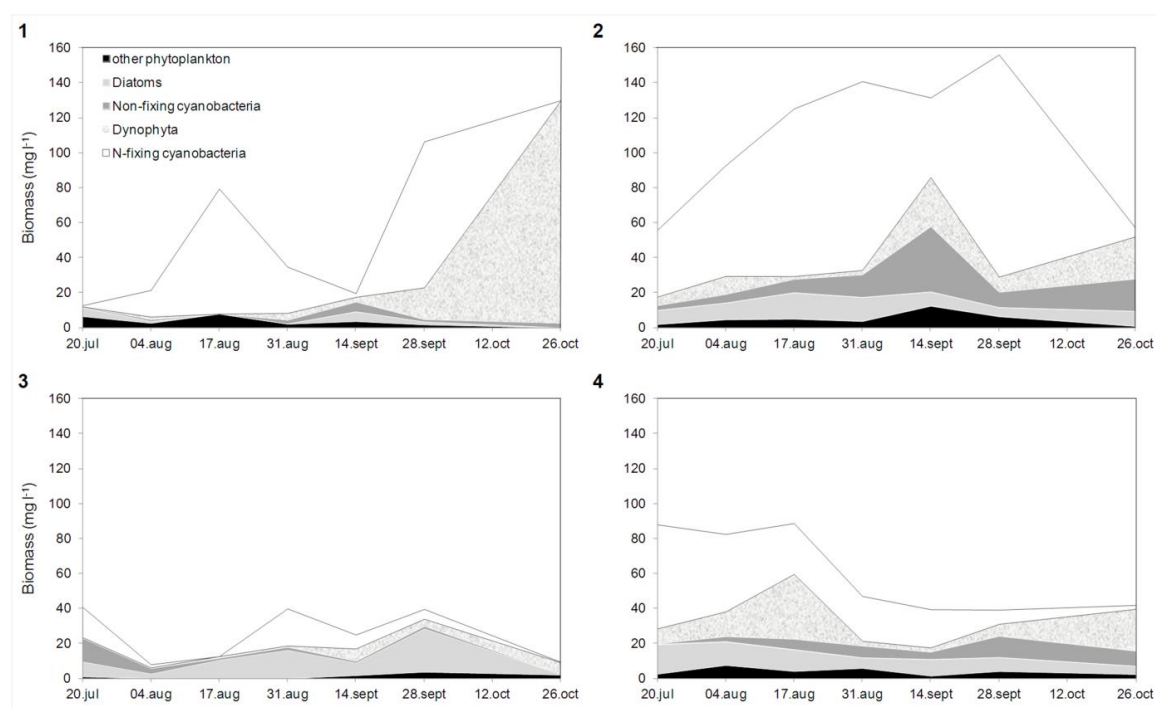
**Table 1** Chemical characteristics of the four sampling sites of the KBWPS in 2009

Sampling sites	Date	$\alpha\text{-Chl}$	$\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}$	Urea-N	SRP	TDP	TP	TDN	TDN/SRP	TN/TP
$\mu\text{g L}^{-1}$											
Western part of Upper reservoir (1)	20.07.	149.7	7.4	6.1	15.7	28.6	50.5	346.4	29.3	1.02	2.57
	03.08.	98.9	80.1	31.8	43.2	53.6	87.2	226.4	155.1	2.89	6.24
	17.08.	221.7	43.8	108.1	123.4	40.7	76.2	267.0	275.3	6.76	7.48
	31.08.	122.8	116.1	45.3	45.9	86.7	120.7	305.0	207.2	2.39	4.54
	14.09.	125.6	75.7	156.4	20.3	31.1	58.2	225.0	252.5	8.12	6.03
	28.09.	110.6	11.5	30.4	29.1	60.8	90.2	221.6	70.9	1.17	6.31
	12.10.	85.7	143.5	105.4	23.8	74.1	105.4	206.0	272.7	3.68	5.50
Eastern part of Upper reservoir (2)	20.07.	163.0	4.5	4.1	13.9	6.4	34.9	222.9	22.5	3.53	9.63
	03.08.	320.2	12.3	3.4	58.3	6.3	39.9	259.6	74.0	11.76	9.79
	17.08.	318.2	13.3	18.2	22.6	6.3	128.6	271.4	54.1	8.54	11.44
	31.08.	366.8	12.5	1.3	16.3	7.0	34.9	310.0	30.2	4.34	11.98
	14.09.	333.9	13.2	2.3	17.3	5.2	29.3	266.7	32.8	6.34	12.99
	28.09.	219.4	16.4	3.1	41.9	7.1	36.3	233.7	61.4	8.59	10.23
	12.10.	98.2	362.8	24.2	31.1	12.4	47.3	181.4	418.1	33.84	8.24
Cassette (3)	20.07.	174.5	14.9	9.2	72.7	240.4	268.1	456.6	96.8	0.40	5.17
	03.08.	98.9	33.5	8.7	57.7	289.5	316.9	454.3	99.8	0.34	7.52
	17.08.	177.7	15.4	6.4	27.7	144.6	204.4	366.8	49.5	0.34	4.07
	31.08.	245.4	18.4	3.9	19.9	104.8	156.8	409.1	42.2	0.40	6.26
	14.09.	214.1	164.0	112.6	19.9	41.0	89.7	308.3	296.6	7.23	7.85
	28.09.	151.2	24.9	11.1	50.8	77.2	128.6	322.4	86.8	1.12	4.93
	12.10.	185.0	445.5	102.3	21.9	133.1	180.1	308.3	569.8	4.28	4.85
Lower reservoir (4)	20.07.	301.0	6.6	5.7	25.8	5.9	40.0	273.4	38.1	6.41	11.46
	03.08.	315.2	16.6	9.6	59.0	8.8	52.2	299.1	85.3	9.71	4.73
	17.08.	264.0	13.8	5.9	31.5	6.5	50.5	267.1	51.1	7.89	11.42
	31.08.	258.5	225.4	30.8	22.9	8.6	50.5	303.7	279.1	32.60	10.18
	14.09.	193.8	323.4	90.3	45.2	5.7	39.3	336.9	458.9	81.05	7.72
	28.09.	198.4	392.6	102.5	41.5	5.8	38.6	209.3	53.6	93.32	11.23
	12.10.	87.1	223.6	84.4	83.9	5.5	34.7	169.6	392.0	71.17	11.64

$K_d$  light attenuation coefficient,  $\nu$   $\text{N}_2$  fixing rates,  $I_k$  light dependence parameter of fixation

## Phytoplankton species composition

At all sampling sites, the phytoplankton was characterized by cyanobacterial dominance (Fig. 2). The water temperature in the summer was high (around 25°C), favourable for the growth of cyanobacterial blooms, which started at the end of the spring, and became dominant in July. All sampling sites except Ingói-berek displayed two N-fixing cyanobacteria peaks, decreasing at the end of September. This observation coincided with relatively high TN:TP and TDN:SRP mass ratios in late autumn, however only a very weak correlation was found ( $r^2 = 0.1842$ ) with the total mass ratios in general, and no correlation was found with the soluble nutrient ratios ( $r^2 = 0.0602$ ). The disappearance of filamentous cyanobacteria species in late autumn rather connects to the decreasing water temperature (20°C) and the increasing wind speeds. *Aphanizomenon flos-aquae* was almost the only N<sub>2</sub>-fixing cyanobacteria species present at the first and third sampling sites, and its contribution to the total phytoplankton biomass at these sites exceeded 70% (and varied between 5–90% at the second and fourth sites). At the second and fourth sampling sites, the phytoplankton community was more diverse. Eight N-fixing cyanobacteria species were identified, including *Anabaena spiroides* (KLeb.), *Anabaena flos-aquae* ((L.) Ralfs), *Aphanizomenon issatchenkoi* ((Ussatzew.) Proschkina-Lawrenko), and the biomass was dominated by *Cylindrospermopsis raciborskii* ((Wolosz.) Seenayya et Subba Raju). The contribution of this alga to the total phytoplankton biomass was generally greater than 70% during the summer and decreased by half as of mid-September. In October, the amount of cyanobacteria species present decreased significantly, and the Dynophyta species and the Diatoms became dominant.



**Fig.2** Phytoplankton biomass and species composition.



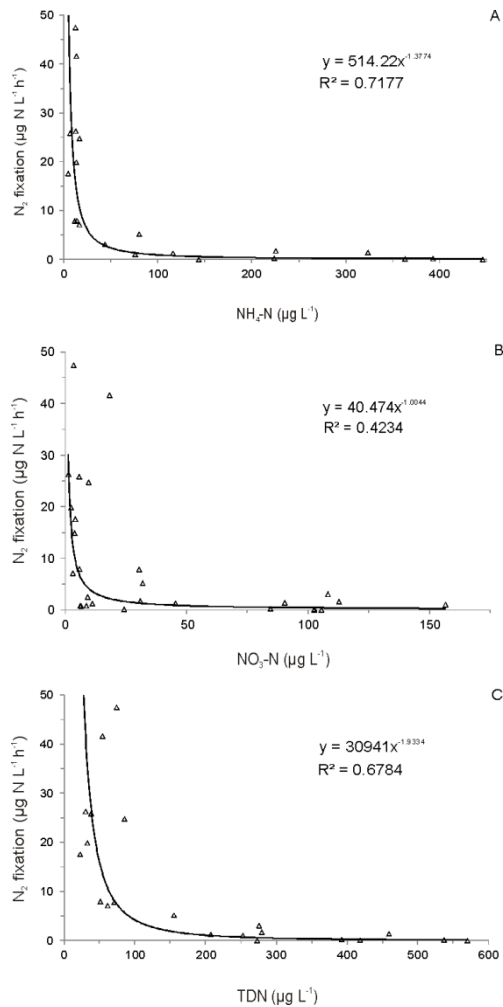
## Phytoplankton nitrogen fixation and its contribution to nitrogen demand

The results of the nitrogen fixation experiments are shown in Table 2. The nitrogen fixing rates and their maxima differed greatly between sampling sites, as well as by date. The dynamics of the chl-*a* related nitrogen fixing rates and the water volume related nitrogen fixing rates were similar. We observed increasing rates at the first sampling site at the beginning of the study period, and at the end of the summer the rates began to decrease. The maximum nitrogen fixation rate at the first site was measured at the end of September ( $7.24 \mu\text{g N L}^{-1} \text{ h}^{-1}$ ), when the  $\text{N}_2$ -fixing cyanobacterial biomass was the greatest ( $\sim 80\%$  contribution to the total phytoplankton biomass). However, the largest nitrogen fixation ( $40.05 \text{ mg N m}^{-2} \text{ d}^{-1}$ ) was measured here in early August. The contribution of nitrogen fixing cyanobacteria to the algae N-demand was insignificant (between 0.5 and 6.33%) (Table 2). A similar trend was apparent at the second sampling site, but with much larger rates (generally two orders of magnitude higher). The greatest nitrogen fixation was measured in August ( $364.7 \text{ mg N m}^{-2} \text{ d}^{-1}$ ). Thereafter, nitrogen fixation and its contribution to the algae nitrogen demand decreased into autumn ( $\sim 30\%$ – $0.08\%$ ). At the beginning of the study period, the nitrogen fixing rates were low in the Cassette, and at the end of August were one and sometimes two orders of magnitude larger than dates before or after. The contribution of nitrogen fixation to the phytoplankton nitrogen demand was negligible during the whole period (generally  $<2\%$ ; max.  $9\%$ ), despite the relatively large  $\text{N}_2$ -fixing cyanobacterial biomass ( $\sim 55\%$ ). At the fourth sampling site, the nitrogen fixing rates declined significantly from July till October ( $28.33$ – $0.257 \mu\text{g N L}^{-1} \text{ h}^{-1}$ ), and resulted in a decreasing trend of the contribution to the total nitrogen supply ( $18\%$ – $0.07\%$ ). The maximum nitrogen fixation ( $213.8 \text{ mg N m}^{-2} \text{ d}^{-1}$ ) was observed in the middle of July, two weeks earlier than at the second sampling site. On the basis of the calculated primary production, the contribution to the total nitrogen supply was  $18.5\%$ .

The effect of different dissolved N-forms (Fig. 3a and b) and of total dissolved nitrogen (TDN) (Fig. 3d) on the rate of  $\text{N}_2$ -fixation was examined. Ammonium showed a strong correlation ( $r^2 = 0.7177$ ) with the  $\text{N}_2$ -fixation, while nitrate showed a much weaker correlation ( $r^2 = 0.4234$ ). Urea was generally found in the largest proportion of the soluble nitrogen forms in the water, and it did not seem to affect the  $\text{N}_2$ -fixation. However, a relatively strong correlation between TDN and  $\text{N}_2$ -fixation was also found ( $r^2 = 0.6784$ ).

**Table 2** The light conditions, estimated primary production, nitrogen fixation rates, daily fixation and its contribution to the nitrogen supply of algae in the KBWPS

Sampling site	Date	$K_D$ $m^{-1}$	Secchi cm	$I_K$ $\mu M\ m^{-2}h^{-1}$	$v$ $\mu g\ L^{-1}h^{-1}$	$N_2$ fixation $mg\ N\ m^{-2}day^{-1}$	primary production $mg\ C\ m^{-2}\ day^{-1}$	contribution of $N_2$ fixation %
Western part of Upper reservoir (1)	20.07.	8.917	20	84.78	0.545	4.275	4424	0.55
	03.08.	6.406	27	62.43	5.270	40.050	3591	6.33
	17.08.	7.714	27	49.44	2.650	18.612	5785	1.83
	31.08.	7.754	23	138.80	1.323	8.670	3706	1.33
	14.09.	7.833	22	76.81	0.893	3.264	3490	0.53
	28.09.	5.857	32	89.60	7.237	28.210	3232	4.96
	12.10.	-	-	-	0.016	0.072	2610	0.02
Eastern part of Upper reservoir (2)	20.07.	4.423	28	62.35	19.060	207.852	4573	25.81
	03.08.	7.169	27	78.03	50.990	364.700	7230	28.64
	17.08.	7.573	22	125.01	41.410	209.270	7326	16.22
	31.08.	9.746	20	84.05	27.890	199.420	7483	15.13
	14.09.	9.591	20	73.34	18.980	84.800	6390	7.54
	28.09.	6.843	28	97.61	8.080	43.110	5092	4.81
	12.10.	-	-	-	0.064	0.387	2834	0.08
Cassette (3)	20.07.	6.588	45	71.63	2.673	17.790	4985	2.03
	03.08.	7.173	31	73.11	0.794	4.532	3665	0.70
	17.08.	6.556	35	72.37	0.811	4.570	5256	0.49
	31.08.	7.101	28	-	14.862	74.500	5917	8.27
	14.09.	9.751	25	-	1.058	7.995	4933	1.07
	28.09.	8.692	27	-	1.247	4.950	3993	0.91
	12.10.	-	-	-	0.010	0.040	4164	0.01
Lower reservoir (4)	20.07.	6.935	25	93.40	28.330	213.770	6940	17.49
	03.08.	8.516	20	99.82	25.430	147.200	7501	11.14
	17.08.	9.756	20	60.71	9.690	60.910	6574	5.26
	31.08.	8.702	22	64.35	2.135	16.350	6218	1.49
	14.09.	7.728	28	58.99	1.468	9.370	4643	1.15
	28.09.	5.374	31	99.22	0.280	3.060	4811	0.36
	12.10.	-	-	-	0.257	0.334	2635	0.07



**Fig.3** The effect of different nitrogen forms on the  $N_2$ -fixation.

#### External N-load

The yearly external nitrogen load calculated from the daily water discharge and nutrient concentrations is shown in Table 3. Average monthly external N load by inflows and  $N_2$ -fixation were compared. The annual trend of external nitrogen loading is characterized by a winter maximum (January to March) and a summer minimum (July to September), which corresponds to the amount of runoff from the Zala River. Nitrogen fixation played an important role in nitrogen loading to the reservoir in summer months due to the low external nitrogen load. Clear differences were found in the fixed nitrogen content between months. In July and August this amount was more than 165 tons over the whole reservoir, which was three times higher than the external N-load. In September and October, the fixed nitrogen content was only 21 tons. From July to October the cyanobacteria fixed ~ 195 tons nitrogen (168 tons in the Upper reservoir, 19 tons in the Lower reservoir, and 5.6 tons in the Cassette). This represents a large share of the annual external nitrogen loading to the lake (~25%).

**Table 3** The external N load to the KBWPS (from Zala River, small inflows and nitrogen fixation) in 2009

Date	Zala River runoff	Zala River	Upper reservoir	Lower reservoir		Zala+ Upper+Lower reservoir	Fixed N <sub>2</sub>
	10 <sup>6</sup> m <sup>3</sup>			Ingói-berek	Marshy field		
				t			
January	40.41	64.32	6.64	1.23	25.00	100.59	-
February	25.93	171.04	12.53	22.83	41.80	237.96	-
March	13.75	65.94	5.82	19.72	32.80	114.20	-
April	9.94	37.26	1.24	6.79	7.70	54.26	-
May	5.92	17.32	0.60	0.86	2.70	24.10	-
June	5.08	15.00	2.35	0.56	3.40	24.34	-
July	6.80	21.74	1.05	0.69	5.60	32.02	40.64
August	3.57	10.95	0.36	0.55	0.40	18.75	130.98
September	3.05	7.88	0.42	0.59	5.00	16.19	21.72
October	4.34	11.68	0.57	1.52	4.30	20.25	0.08
November	6.35	17.09	1.61	5.21	5.60	28.51	-
December	9.17	30.75	4.38	6.93	19.30	58.55	-
Σ of the year	104.30	470.95	37.58	67.59	153.50	729.72	193.43

## Discussion

The KBWPS is an extremely shallow biological water purification lake-system. The selected study sites are characterized by different features (e.g. available nutrient content, retention time, species composition, and phytoplankton biomass), and thus allowed the study of nitrogen fixation across variable conditions.

Nitrogen fixation is the only process counteracting the effect of the nitrogen loss by denitrification in nature (Tönno & Nöges, 2003). The initiation conditions in shallow lakes are under debate (Flett et al., 1980), but several studies show that low TN:TP mass ratios favour the development of nitrogen fixing (and non-fixing) cyanobacterial blooms (Tönno & Nöges, 2003). Studies assessing the development of cyanobacteria dominance in response to TN:TP ratios are widespread, and the determined ratios vary greatly (5–10; 29) (Schindler, 1977, Seip, 1994, Michard et al., 1996, Bulgakov & Levich, 1999 and Smith et al., 1983). The critical N:P ratio is often close to the Redfield mass ratio (7:1) (Redfield, 1958, Falkowski & Davis, 2004), where the phytoplankton become N-limited and nitrogen fixation occurs (Howarth et al., 1998b). In the KBWPS, the relatively low TN:TP ratios (7–12) suggest high cyanobacterial biomass and intensive nitrogen fixation. N<sub>2</sub>-fixing species were found to comprise almost 60% of the total phytoplankton biomass, but this was relatively weakly correlated with TN:TP mass ratio ( $r^2 = 0.1842$ ). Similarly a very weak correlation was found between nitrogen fixation rates and TN:TP mass ratios ( $r^2 = 0.1358$ ). However, the correlation between the nitrogen fixation rates and the N-fixing cyanobacterial biomass was found to be relatively strong ( $r^2 = 0.684$ ).

According to many authors (Smith et al., 1987, Willén, 1992, Lathrop et al., 1998, Downing et al., 2001), absolute concentrations provide more insight than ratios. However, according to Tönno & Nöges (2003), this does not seem to hold true for very shallow polymictic lakes, such as the KBWPS. In KBWPS, a relatively strong correlation was found between nitrogen fixation and the concentration of total dissolved nitrogen ( $r^2 = 0.6784$ ). This correlation, as well as the high cyanobacterial biomass, also suggested that the concentration of TDN was relatively low, but the ambient concentrations of N-forms did not display nitrogen depleted conditions. Kenesi et al. (2009) found that high  $\text{NH}_4$  content could repress, but not stop the nitrogen fixation in continuous flow cultures of *C. raciborskii*. Such limitation was also found in the KBWPS at the fourth sampling site at the end of August. At this time, the  $\text{NH}_4$  concentration reached its maximum (several hundred  $\mu\text{g L}^{-1}$ ), the  $\text{PO}_4\text{-P}$  and soluble P (TDP) were present in concentrations much higher than their possible limitation concentrations, the N-fixing species comprised more than 50% of the total biomass, and nitrogen fixation remained insignificant. The relatively strong negative correlation between TDN and  $\text{N}_2$ -fixation observed throughout the whole system demonstrates that, in addition to the ratios, the absolute concentrations of nutrients (N and P) are also necessary to estimate the nutrient deficiency and the significance of  $\text{N}_2$  fixation.

It appeared that this shallow, turbid water was light, rather than nutrient limited (see  $K_d$ ), and that the growing conditions of phytoplankton were affected more by light intensity than by the available nutrient concentrations or ratios thereof. Furthermore, the relatively low transparency of the water column (see Secchi depths, Table 2) favoured cyanobacteria which have a lower light requirement than the other species (Présing et al., 1996). The presence of *Aph. flos-aquae* as the only  $\text{N}_2$ -fixing cyanobacteria at two of the sampling sites (first and third) could be related to the concurrence of high concentrations of ammonium and soluble P. These conditions favour the development of this algal bloom over other  $\text{N}_2$ -fixing cyanobacteria (De Nobel et al., 1997). Otherwise, we did not find significant differences between the light dependence parameters and the water temperature between sampling sites. This could highlight the different competitive advantages of *Aph. flos-aquae* and *C. raciborskii* (Kovács et al., 1999), which comprise more than 70% of the total phytoplankton biomass at the two other sampling sites (second and fourth). According to Wetzel (1983), the planktonic nitrogen fixation carried out by cyanobacteria is closely related to the amount of cyanobacterial biomass. Here a strong relationship ( $r^2 = 0.7057$ ) was also found between the  $\text{N}_2$ -fixing cyanobacteria biomass and the amount of nitrogen fixed. However, although the nitrogen fixing rates generally increased with the steadily growing biomass (owing to the growing nutrient demand), at the end of the summer the rates decreased continuously while the N-fixing cyanobacteria

contribution to the total biomass was still relatively high, likely as a result of the moderate N-demand (increasing soluble nitrogen concentration).

In oligotrophic and mesotrophic lakes the contribution of phytoplankton to the nitrogen budget are unimportant (less than 1% of the total N-load). In contrast, in eutrophic lakes this contribution varies between 6–82% (Howarth et al., 1988a). In the KBWPS the N<sub>2</sub>-fixing cyanobacteria fixed a large amount of N<sub>2</sub> over the study period, and its contribution to the total nitrogen supply of the phytoplankton sometimes (July-August) reached 30%, as calculated from the primary production. Comparing external N-loads to the fixed nitrogen content in the Upper reservoir, nitrogen loading by fixation was found to be more than three times higher than the external load from inflows during the summer, whereas in the lower reservoir, nitrogen loading by fixation was 30% of the external load over the same period. During the summer months, the nitrogen fixing cyanobacteria played a key role in addressing the nitrogen demand of phytoplankton. For the 21 km<sup>2</sup> area, the nitrogen fixation contributed approximately 25% to the annual nitrogen load. This amount is ten times higher than that measured by Gorzó (1990) between 1986 and 1989, and is double the amount in 2002 as described by Kovács (2002).

The high proportion of fixed nitrogen in the total nitrogen supply to phytoplankton in summer months, and the increasing trend of the amount of fixed nitrogen in the reservoir indicate a gradual decline in the quality of water entering the western basin of Lake Balaton. To preserve the precedent of good lake water quality, this should be taken into account in the planned extension and operations management of the KBWPS.

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